OSBP as a Prophylactic Antiviral Drug Target: Investigating the Potential for Palindromic Binding of Novel Chimeric Scaffolds

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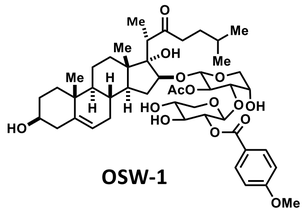
Introduction and Experimental Design

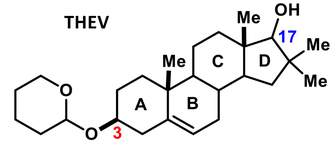
➢ **OSBP as a Prophylactic Antiviral Drug Target**

The role of OSBP in positive-sense ssRNA viral replication has recently been demonstrated. In this role, OSBP is recruited by the viral 3A protein to provide cholesterol to the developing viral replication organelle (VRO). OSW-1, an OSBP high-affinity natural product ligand, has been shown to initiate OSBP degradation via the proteosome and elicits a prolonged repression via a currently unknown mechanism. Prolonged OSBP repression is then able to reduce viral load *in vitro* for several enteroviruses. OSBP is known to bind many different ligands, many of which also target the OSBP homologue ORP4. Although short-term OSBP repression does not show detrimental effects *in vitro*, ORP4 is essential for several cell types. Because of this, more information about ligand binding modalities to OSBP is necessary to determine if this interesting protein can serve as a prophylactic antiviral drug target.



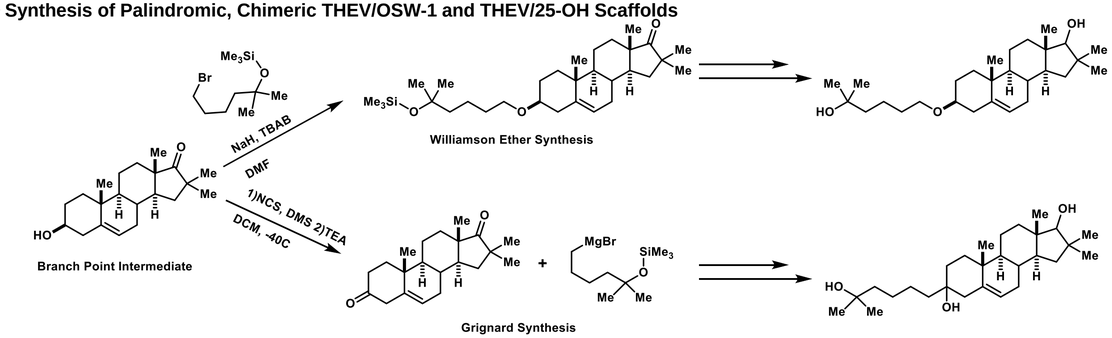
➢ **THEV: A Minor Enviroxime-like Compound**

THEV has been identified as an OSBP ligand with comparable affinity to OSW-1. Although both compounds share a steroid core, they significantly differ in their decorative moieties. The understood OSW-1 binding mode places the A-Ring 3-hydroxy at the bottom of the binding pocket. This binding mode is likely not possible for THEV due to the THP group on the A-ring. We suspect the D-ring 17-OH present on THEV serves the purpose of the 3-OH on OSW-1. This would imply the THEV binding modality is “Palindromic” compared to OSW-1. To assess this hypothesis and further elucidate ligand biding modalities to OSBP, chimeric scaffolds with palindromic assembly will be synthesized and their binding affinity to OSBP will be determined via a radioligand binding assay.

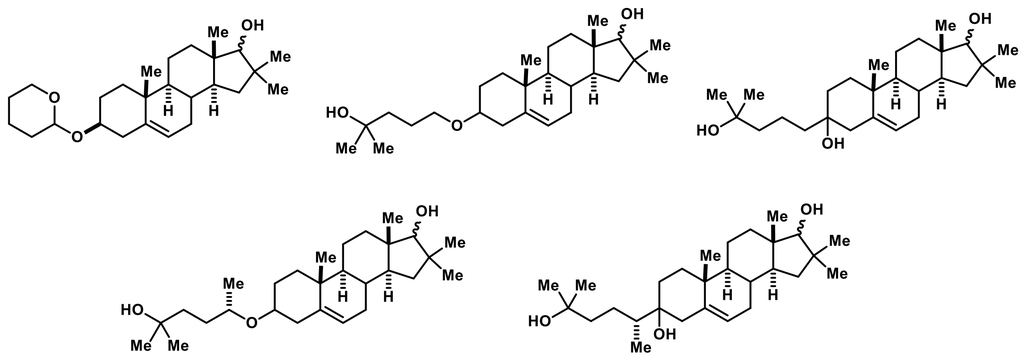


Chimeric Scaffold Synthesis and Design

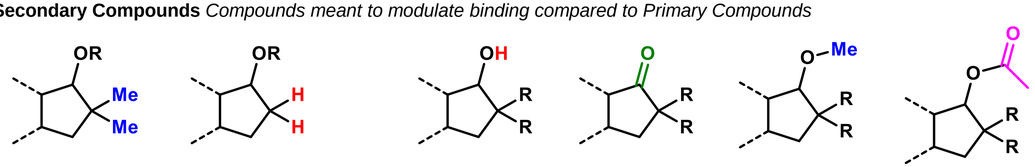
➢ **Synthesis of Palindromic, Chimeric THEV/OSW-1 and THEV/25-OH Scaffolds**



➢ **Target Primary Compounds** *Compounds meant to examine the primary hypothesis*



➢ **Target Secondary Compounds** *Compounds meant to modulate binding compared to Primary Compounds*

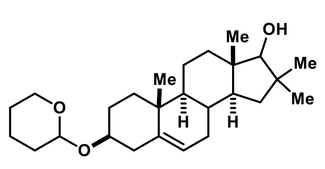


Examination of the effect of Steric Bulk adjacent to hydrogen bonding site

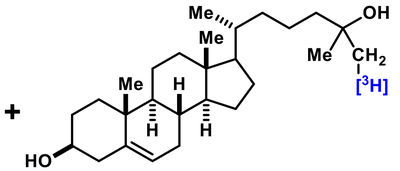
Examination of hydrogen bond donor, acceptor, lower polarity/small bulk, lower polarity/high bulk

Protein-Ligand Interaction: OSBP Binding Studies

➢ **Binding Study Format** *Measuring the interaction of ligand to protein via a Competitive Binding Assay format*



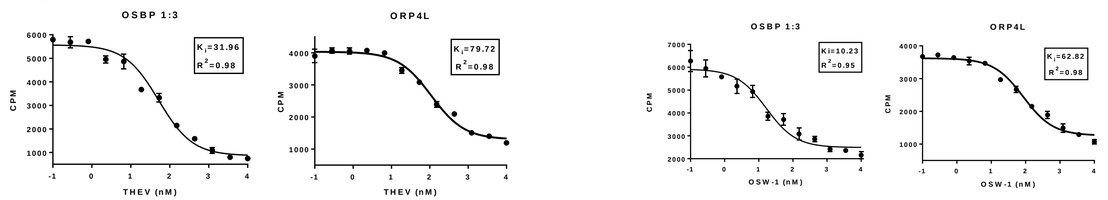
Test Ligand



Measurable Ligand

Tritiated 25-hydroxycholesterol ([^{3}H]-25OH) serves as the Measurable Ligand. 25-OH is an endogenous high-affinity OSBP ligand (K_D=22+/- 5 nM)

Increasing concentrations of Test Ligand are incubated with constant concentration of Measurable Ligand and protein. After incubation, samples are combined with scintillation fluid. Radiation from residual Measurable Ligand causes the scintillation fluid to emit light, which is then detected. If a Test Ligand binding affinity is comparable to or better than the Measurable Ligand, a binding curve can be constructed. The binding curve enables the calculation of a binding constant, which is a normalized value comparable between different test ligands.

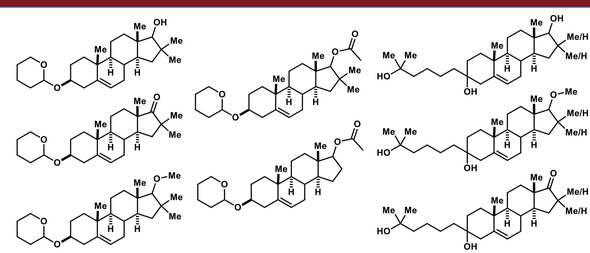


Current Progress and Future Directions

➢ **20 of the current 40 proposed compounds have been synthesized.**

➢ **Binding studies will begin with THEV and its analogues, then continue with additional ligands as synthesis is completed.**

➢ **Future directions may see other biological assay methods employed (e.g. Cytotoxicity) as well as additional ligand development.**



References

1) *Biochem. Pharmacol.*, **2013**, *86*, 89-95;
2) *Nature Chemical Biology*, **2011**, *7*(9), 639–47
3) *Cell Reports*., **2015**, *10*, 600-615